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PERMEABILITY OF MEMBRANES AS RELATED TO THEIR COMPOSITION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 231

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(WITH SIX FIGURES)

Introduction

In a previous paper (1) measurements were made of the rate at which water passes through known areas of certain membranes. Different species of plants and different membranes of the same species showed large differences in the rate of penetration. From these facts the following questions arise. What substances in the membrane are determining the rate at which water can pass through it? Of what relative importance in this process are lipoids, proteins, tannoids, suberin, pectic substances, etc.? An attempt was made to answer these questions by quantitative measurements, and this paper records the results.

The rôle of different substances in the membrane in regulating its permeability to water was determined by comparing the permeability of a membrane before and after extracting it with the solvent of the material studied. Thus, for example, to determine the effect of lipoid materials upon the permeability of the membrane, its permeability in the normal condition was first measured; it was then extracted with a lipoid solvent (for example, acetone), and its permeability again measured. These two measurements were then compared. To accompany these measurements, microchemical tests were made to give information as to the nature of the materials composing the membrane, and the effect of these extractions upon its composition; and for comparison chemical analyses were made of the membrane and the extracted materials.

Methods

The osmometer used has been described and figured in a previous paper (1). The apparatus was immersed in a Freas constant temperature water bath and the temperature kept constant to approximately 0°.1 C. In all experiments reported in this paper the temperature was 25° C. Sodium chloride solutions were used to induce the osmotic movement of water through the membrane. With the peanut membrane (*Arachis hypogaea*), which is rather highly permeable, concentrations of 0.5 M or 0.6 M were found to be suitable; but for squash, cocklebur, etc., which have fairly high resistance to water penetration, strong solutions of sodium chloride were necessary; for use with such membranes a saturated solution was used, that is, saturated at room temperature.

As noted in the previous paper, different membranes of the same species differ in their permeability to water. This difficulty was overcome by using the same membrane under the various conditions of the experiment. In the tables of results each line of a table represents data obtained from a single membrane; it is not possible to compare one membrane with another; the data given by an individual membrane under different conditions must be compared.

Effect of extracting membranes with boiling water

It was noted that heating the seed coat of *Arachis hypogaea* in boiling water increased the rate at which water passed through. Measurements were made to determine the extent of this increase; and other membranes were tested for similar behavior. The method employed was as follows. A membrane was removed from a soaked seed, fitted into the osmometer, and a measurement made of its permeability. It was then removed from the apparatus, placed in cold distilled water, and heated to boiling point and allowed to remain in boiling water for 5 minutes. It was then placed in the osmometer and a reading made of its permeability. This process was then repeated with other membranes. Table I shows the results, which are expressed in milligrams of water passing through a membrane per hour; the same unit is also used in subsequent tables.

The data show that heating the membrane in boiling water increased the permeability of the peanut and almond membranes, but not that of the grapefruit and squash coats. However, the last two membranes are so slightly permeable that a considerable increase in permeability might escape notice. With more delicate methods an increase under these conditions might appear. In heating the peanut seed coat a brownish extract was obtained, and it is thought that the increase was due in a large measure to the removal of the soluble substances. The nature of these substances will be treated in that portion of the paper dealing with microchemical and chemical tests.

TABLE I

EFFECT OF EXTRACTING MEMBRANES WITH BOILING WATER

Membrane	Area in sq. mm.	Concentration of solution	Rate before extraction	Rate after extraction	Percentage of increase
Peanut................	19.635	0.5 M	28.31	66.73	135.7
"	"	"	32.35	82.90	156.3
"	"	0.6 M	35.05	115.59	229.8
"	50.265	0.5 M	89.54	212.31	137.2
"	19.635	0.6 M	54.67	150.00	174.4
"	50.265	0.5 M	34.67	114.30	229.7
"	"	"	88.56	210.00	137.1
"	19.635	0.5 M	40.44	101.10	150.0
Almond...............	"	Saturated	17.52	105.14	500.1
Grapefruit............	"	"	0	0	....
"	"	"	0	0	....
Squash................	"	"	0	0	....
"	"	"	0	11.76	....

TABLE II

EFFECT OF HEATING DRY MEMBRANES

Membrane	Area in sq. mm.	Concentration of solution	Rate before heating	Rate after heating	Percentage of increase
Peanut................	19.635	0.5 M	28.31	42.47	50.03
"	50.265	0.8 M	78.86	101.10	28.2

To gain further information on this point, the effect of heating the dry membrane was compared with the effect of heating the membrane in water. The permeability of a membrane was first measured; it was air dried, placed in a glass tube, and the latter heated in water as described. The membrane was then soaked in water, placed in the osmometer, and a reading again made of its permeability. Table II expresses these results.

The results indicated in table II show that part of the increase in permeability after heating in water is due to the heating effect itself, and that not all the increase is due to the extraction of materials from the membrane.

Effect of extracting membranes with hot lipoid solvents

The rôle of lipoid substances present in the membrane in limiting the rate of penetration of water was investigated by determining the permeability of a membrane before and after extraction of the membrane with a lipoid solvent. The solvents used were alcohol, acetone, and ether. After 4 hours' treatment with the solvent, the solvent was removed from the membrane and the latter soaked in water before being placed in the osmometer. Table III shows the results obtained. In this series all membranes were previously heated in boiling water. It will be noted that extracting the membrane with lipoid solvents greatly increased the permeability to water. Only in the case of the grapefruit seed did such treatment fail to show an increase. The different solvents did not

TABLE III

EFFECT OF EXTRACTING MEMBRANES WITH HOT LIPOID SOLVENTS

Membrane	Area in sq. mm.	Concentration of solution	Rate before extraction	Rate after extraction with hot alcohol	Percentage of increase	Rate after extraction with hot acetone	Percentage of increase	Rate after extraction with hot ether	Percentage of increase
Peanut	50.265	0.5 M	92.67	144.45	55.9	......	......	169.84	83.3
"	19.635	0.6 M	52.23	92.34	76.8	......	......	92.47	77.0
"	50.265	0.5 M	98.40	228.48	132.2	......	......	......	......
"	"	"	78.86	161.76	112.6	171.87	126.3	......	......
"	"	"	151.65	257.80	69.9	......	......	279.06	84.1
"	19.635	"	101.10	117.26	15.9	......	......	131.43	30.0
"	50.265	"	113.24	242.65	114.3	217.70	92.2	......	......
Squash	19.635	Saturated	11.12	99.42	793.9	101.10	809.2	......	......
"	"	"	15.51	82.90	434.5	98.07	532.6	......	......
"	"	"	11.45	111.22	871.3	119.08	939.8	......	......
Almond	50.265	"	60.66	232.55	283.4	234.89	287.2	......	......
"	"	"	50.55	279.71	452.3	279.06	452.0	......	......
Cocklebur	19.635	"	20.22	48.28	138.8	40.44	200.0	......	......
"	"	"	14.83	45.83	209.1	......	......	......	......
"	"	"	22.24	56.62	154.6	......	......	......	......
Grapefruit	"	"	0	0	......	......	......	......	......
"	"	"	0	10.11	......	......	......	......	......
"	"	"	0	0	......	......	......	......	......

show large differences, but in general acetone and ether were more effective than alcohol in increasing the rate, possibly due to their better dissolving power. The effect of the lipoid solvents upon the permeability of the squash membrane is to be noted especially. Most squash membranes will not show any penetration by water under the conditions of these experiments until they are treated with a lipoid solvent. They then become rather permeable to water.

Effect of extracting membranes with cold lipoid solvent

The significance of lipoids in the membranes is well shown by the series of measurements made when the extraction was carried on at room temperature. The unheated membranes were merely placed in bottles of acetone and allowed to undergo extraction at room temperature for 18 hours. The permeability of the membranes was measured before and after such treatment. Table IV shows the results of this experiment.

TABLE IV

EFFECT OF EXTRACTING MEMBRANES WITH COLD LIPOID SOLVENT

Membrane	Area in sq. mm.	Concentration of solution	Rate before extraction	Rate after extraction with cold acetone	Percentage of increase
Peanut	19.635	0.5 M	14.49	60.66	318.6
"	50.265	"	65.71	246.68	275.4
"	19.635	"	36.00	88.29	145.3
"	"	"	24.27	76.84	216.6
Pumpkin	50.265	Saturated	0	251.77	....
"	"	"	4.04	141.54	....
Squash	19.635	"	0	118.64	....
"	"	"	0	107.84	....
Almond	"	"	17.52	34.37	96.18
"	50.265	"	101.10	153.67	53.21
Grapefruit	19.635	"	0	0	....

Infiltration experiments

Experiments were made to infiltrate the extracted membrane with the extracted material. Membranes that had been extracted were soaked in the solvent containing the lipoid extract and then exposed to evaporation; by a continuance of this process it was hoped to impregnate the membrane again with the material that had previously been removed. Table V shows these results. It is apparent that while the permeability is reduced by the infiltration, the decrease does not reach the low point exhibited by the membrane before the original extraction. Apparently the lipoid materials cannot be put back into the membrane in the condition and position in which they existed before extraction. This would indicate an organized distribution of these materials in the membranes in nature.

TABLE V

EFFECT OF INFILTRATING MEMBRANES

Membrane	Area in sq. mm.	Concentration of solution	Rate before extraction	Rate after extraction with hot acetone	Rate after extraction with cold acetone	Rate after infiltration with lipoid extract
Peanut...........	50.265	0.5 M	65.71	....	246.68	181.98
"	19.635	"	24.27	....	76.84	30.33
Pumpkin..........	"	Saturated	4.08	146.26	....	84.92
Cocklebur.........	"	"	16.17	54.59	....	42.46
Almond...........	50.265	"	101.10	298.26	....	232.52
"	"	"	121.34	326.03	....	258.81

Effect of extracting membranes with calcium chloride

The calcium ion has been shown by HANSTEEN-CRANNER (2) to reduce the rate of water intake by the cell walls of roots, and it was thought desirable to determine whether calcium had similar effects upon the permeability of seed coats to water. Membranes were selected and measurements made of their permeability to water; these membranes were then soaked for 24 hours in calcium chloride solutions of the concentrations described later; the membranes were then rinsed in distilled water, placed in the osmometer, and their permeability again measured. Table VI shows the results. It thus seems that calcium chloride at the concentrations used increased the permeability of the membrane to water. Whether more dilute concentrations would have similar or opposite effects is yet to be determined.

The fact that callose is soluble in calcium chloride solutions would suggest the hypothesis that the increase in permeability is due to the extraction of callose from the membrane. No microchemical differences were noted in membranes before and after extraction in calcium chloride, however. The peanut seed coat became dark brown in color after treatment in saturated calcium chloride.

TABLE VI

EFFECT OF EXTRACTING MEMBRANES WITH CALCIUM CHLORIDE

Membrane	Area in sq. mm.	Concentration of solution	Rate before extraction	Rate after extraction	Percentage of increase	Concentration of CaCl
Peanut	50.265	0.5 M	89.17	117.96	32.3	Saturated
English walnut	19.635	Saturated	22.92	38.43	67.6	"
Almond	"	"	24.26	48.53	100.0	"
"	"	"	26.96	32.69	21.3	1 gm. in 259 cc. water
Peanut	50.265	0.5 M	205.58	220.06	7.0	1 gm. in 259 cc. water

Microchemical tests

The membranes were examined microchemically to determine the nature of the substances composing them, and to note the effect upon them of the various methods of treatment. Thin cross-sections of the seed coats were made with a freezing microtome. These sections were 12.5–25 μ thick. The substances for which tests were made are given, together with the tests applied in each case. The textbooks of TUNMANN (6) and of MOLISCH (4) were consulted for directions regarding these tests.

Tests for suberin.—Insoluble in 50 per cent chromic acid and concentrated sulphuric acid; soluble in 3 per cent alcoholic potash after heating; gives the ceric acid reaction; and stains red with Sudan III.

Tests for tannins.—Blue color with dilute ferric chloride, and a violet color with 0.1 per cent gold chloride.

Tests for lipoids.—Soluble in acetone, ether, and hot alcohol; stain red with Sudan III; true fats give crystals when saponified with potassium hydroxide.

Tests for pectic substances.—Stain red with ruthenium red, and dissolve by treatment with 2 per cent hydrochloric acid followed by 2 per cent potassium hydroxide.

Tests for proteins.—Biuret reaction; those containing tyrosine give a red color with Millon's reagent; those containing tryptophane give a violet color by Liebermann's reaction; other protein tests were not found to be suitable because the color of the membranes interfered with the tests.

Tests for cellulose.—Blue color with sulphuric acid and iodine, and dissolves in copper-oxide-ammonia.

Membrane of peanut (*Arachis hypogaea*)

The cells in the layer at *a* (fig. 1) have walls of cellulose and pectin, and their contents consist of granular lipoid substances. The layer at *b* contains coloring matters, of which tannin was shown to be one. A water extract of this seed coat also gives a positive reaction for tannin. The color in this layer is not completely removed either by treatment with hot water or with lipoid solvents. The layer at *c* consists of the old walls of a parenchymatous tissue that, in the mature seed coat, is much compressed. These walls are of cellulose and pectin. It requires a long period of heating in acid and dilute base to cause these cells to fall apart. The membrane at *d* has no visible contents. The outer walls are of cellulose and pectin, but of a type very resistant to chromic acid. When sections of this membrane are treated with chromic acid these walls are the last to be broken down. Furrows extend across the thick wall of this layer, but the remains of protoplasmic connections between the cells could not be detected. Fatty bodies could be seen

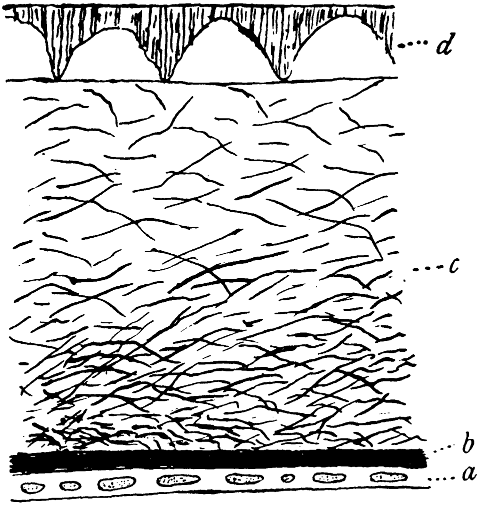

FIG. 1.—Cross-section of seed coat of *Arachis hypogaea*, ×450.

occasionally in the layers *c* and *d*, but saponification crystals were not obtained in place in the tissue.

That lipoid substances are present in the peanut membrane is shown by the analysis of the membrane given later in this paper. It is probable that the lipoids are present in a very fine state of dispersion. No distinctly suberized layers could be detected in the seed coat. All protein tests were likewise negative.

The hot water extraction removes most of the extractable tannins from the membranes, also part of the lipoid materials; the increase in permeability by heating the membrane in boiling water is probably due in large measure to the removal of these substances; and the increase in permeability after extraction with acetone, alcohol, etc., is due to the further removal of lipoid materials. The high permeability of the peanut membrane as compared with other membranes studied is related to its low lipoid content, and especially to the lack of the layers of cells filled with lipoid substances that were found in the other seed coats.

Membrane of cocklebur (*Xanthium pennsylvanicum*)

The walls at *a* (fig. 2) are thick and lignified; those at *b* are much compressed and are composed of cellulose and pectin; at *c* is a suberized layer containing tannins. Tannins also appear in *b*, but may have diffused there from *c*. The layer at *d* and *e* is an endosperm layer; the cell walls marked *e* are composed of cellulose and pectin, and the contents *d* are of lipoid substances. Extraction with lipoid solvents does not completely remove the fatty substances from this layer. Protein tests all gave negative results. Layers *a* and *c* are resistant to chromic acid, but layer *d*, *e* breaks up, and the fatty substances flow out in drops.

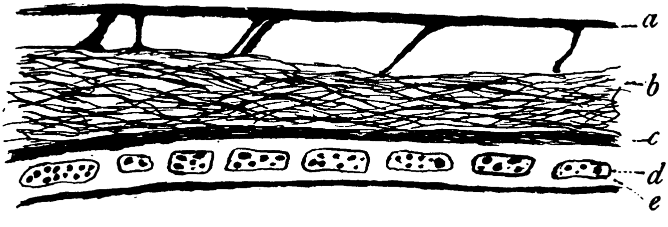

FIG. 2.—Cross-section of seed coat of *Xanthium pennsylvanicum*, ×450

Membrane of squash (*Cucurbita maxima*)

This is the greenish membrane surrounding the cotyledons, and is obtained from soaked seeds by first removing the horny white spermoderm layer, and then removing the greenish coat from the cotyledons. At *d* (fig. 3) is the greenish layer; at *o* is a compressed layer of the cell walls composed of cellulose and pectin. The inner layer is the endosperm layer, and the cells of this layer are completely filled with fatty substances. The walls are thin and are of cellulose and pectin. In some cases protein reactions were positive in layer *d*, but most tests were negative. No tannins were found and suberized membranes were absent.

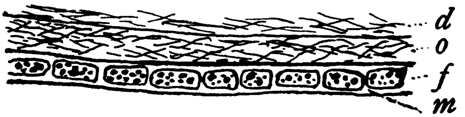

FIG. 3.—Cross-section of seed coat of *Cucurbita maxima*, ×450.

It is believed that the high resistence to the flow of water through this membrane is due to the high content of lipoid substances in layer *f*, *m*. These substances are almost entirely removed by treatment with lipoid solvents, and a large increase in permeability results. It is worthy of note that although this membrane is highly resistant to the passage of water through it as compared with other membranes, distinctly suberized layers do not enter into its composition.

Membrane of almond (*Prunus Amygdalus*)

Large cells *f* (fig. 4) were found to extend from the outer surface of the seed coat. These were in some places much distorted in shape and they also did not cover the entire surface. The walls were of cellulose and pectin, and protein tests gave positive results in this layer. At *d* is a thick layer of parenchymatous cell walls giving cellulose and pectin reactions. A suberized layer is found at *e* and tannin reactions were positive in this region. At *m* again is a layer of compressed parenchymatous cell walls. The endosperm layer is at *o*, *s*. The cells are filled with fatty substances (*o*), and the cell walls *s* are composed of cellulose and pectin. These walls are not thick as compared with either the *Citrus* or *Xanthium* coats. The cell contents also are not completely removed by extraction with lipoid solvents.

Membrane of grapefruit (*Citrus grandis*)

Layer *f* (fig. 5) is a mucilaginous mass of cell wall material giving both cellulose and pectin reactions. The slimy character of soaked seeds of this species is due to this layer. At *o* is a thick suberized layer impregnated with tannin and other coloring materials. The inner wall of this layer is especially well suberized. It is more resistant to chromic acid than any layer in any other membranes studied. The thick endosperm layer is marked at *d* and *w*. In some places this layer is 2 cells and in other places only 1 cell thick, the walls are very thick and are composed of cellulose impregnated with pectin. They break apart on treatment with

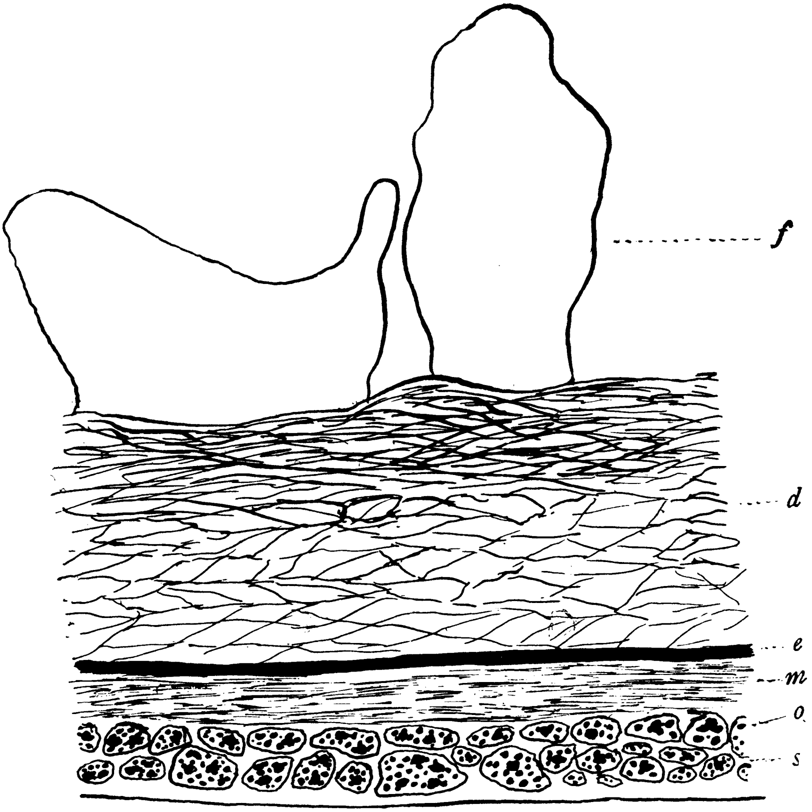

FIG. 4.—Cross-section of seed coat of *Amygdalus communis*, ×450

dilute acids and bases. The cells themselves are filled with fatty substances that are only partly removed by extraction with lipoid solvents. Even after 18 hours of extraction lipoids may still be detected in the coat.

In these various experiments the grapefruit seed coat differed from all others in failing to show increased permeability, or at least a readable rate of water movement after any of the methods of extraction. All tests of this membrane with the osmometer indicated a relatively high resistance of the coat to the passage of water.

A parallel experiment was carried out to give further evidence on this point. This was done by comparing the absorption of water by the seeds, one lot with coats and the other lot without coats. More than one embryo is usually found in these seeds, so that when the coat is removed the embryos fall apart and accurate weighings are impossible. To overcome this difficulty, a narrow band of seed coat was left around the embryos. This band held the embryos together so that weighings could be made. Table VII shows the results obtained, and curves of these results are shown in fig. 6.

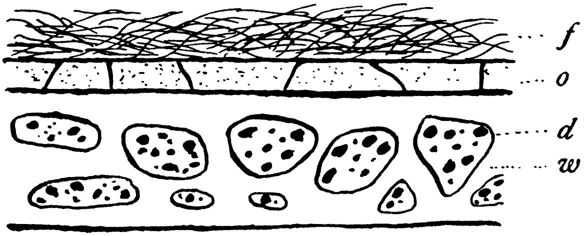

FIG. 5.—Cross-section of seed coat of *Citrus grandis*, ×450

It was found that treating the grapefruit membrane with acids and bases increased its permeability to such an extent that a readable rate of water movement could be obtained. Thus, soaking a membrane in warm 2 per cent sodium hydroxide increased the rate from 0 to 24.26 mg. of water per hour; and in another test soaking the membrane in 2 per cent sodium hydroxide for 24 hours increased the rate from 0 to 72.12 mg. By such treatment, however, the tissue of the seed undergoes a disintegration, so that the outer yellowish layer *o* (fig. 5) comes off. On using this as a membrane the rate was now found to be 210.27 mg. per hour. This indicates that the inner portion of the membrane is the one that offers the resistance to the passage of water, and it is believed that this resistance is due to the presence of the large amount of fatty substances surrounded by thick pectinized walls.

TABLE VII

RATE OF ABSORPTION OF WATER BY SEEDS OF *Citrus grandis*

Time	COATS ON		COATS OFF	
	Weight in gm.	Percentage of water imbibed	Weight in gm.	Percentage of water imbibed
Commencing	2.423	6.13	2.087	5.67
After 12 hours	2.762	20.9	3.135	58.9
" 36 "	2.888	26.5	3.233	64.3
" 84 "	2.932	28.4	3.171	60.8
" 108 "	2.942	28.9	3.123	54.8
" 144 "	2.986	30.8	3.129	58.7
" 204 "	3.056	33.9	3.139	59.2
Oven dry	2.283	....	1.971	....

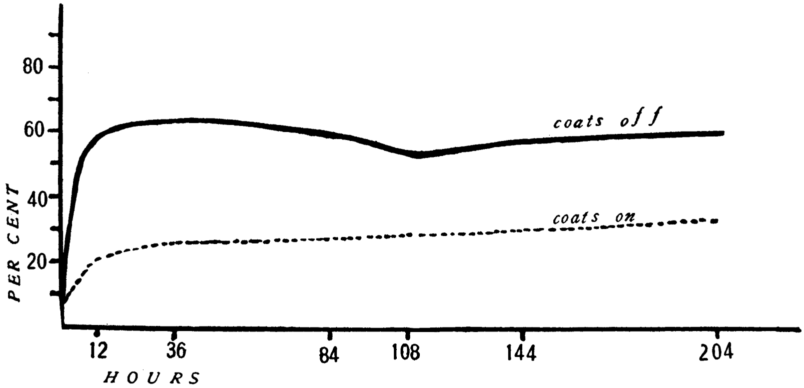

FIG. 6.—Showing rate of imbibition of water by seed of *Citrus grandis;* rate with coats compared with rate without coats; curves obtained by plotting data given in table VIII.

Results of chemical analysis

An analysis was made of a quantity of the seed coats of *Arachis hypogaea.* A complete analysis for all constituents of the membrane was not attempted, but an examination was made only for those substances that appeared to be significant as judged by the results of the extraction experiments and of the microchemical tests.

About 10 pounds of unroasted peanuts were shelled, the pods discarded, and the seeds put at once into cold water to soften the seed coats; as soon as the coats were moist enough, they were removed from the embryos, and dried on blotting paper in the air. The water in which the seeds had been soaking was brownish in color; it was made up to a volume of 1000 cc. with distilled water; this was called the "preliminary extract," and later its content of tannins was determined.

The air dried seed coats were divided into two lots, one lot to undergo extraction in a manner comparable to the extractions performed in the experimental work, and the other lot held as a check to determine the original composition of the membrane before extraction. The first lot was extracted with water, by putting the membranes into cold water, raising the temperature of the water to boiling point, and holding it at this temperature for 5 minutes; the water was then drained off through a filter, and the residue washed with cold distilled water until only a faint straw-colored liquid passed through the filter. The filtrate was made up to 1500 cc., and was called the "hot water extract." The residue was vacuum dried, placed in Schleicher and Schüll extraction cups, and extracted in a Soxhlet extractor for 4 hours with acetone. The extract was made up to a volume of 250 ccm. and was called the "acetone extract." The insoluble material was called "residue."

Methods

Tannins were estimated by Proctor's modification of Lowenthal's method (7, p. 150). The distribution of tannins in the preliminary extract, in the hot water extract, and in the acetone extract was determined.

The lipoids in the acetone extract were estimated by removing a sample, evaporating in a Jena glass dish, and drying to constant weight in a desiccator. To obtain the amount of lipoid in the hot water extract a sample was taken; strips of filter paper were dipped in the sample until the liquid was absorbed by the filter paper; the latter was then dried in a vacuum desiccator, placed in extraction shells, and extracted with acetone in a Soxhlet extractor; the weight of the lipoid material was determined by evaporation. The nitrogen content was measured by the Kjeldahl method, and the protein content by the Van Slyke method, both of the latter methods as described by MATHEWS (3). An attempt was made to determine the phosphorus content of the various portions, but it was found to be very low, and insufficient material was left for conclusive analyses. The results of the analyses are summarized in table VIII.

TABLE VIII

RESULTS OF ANALYSIS OF SEED COATS OF *Arachis hypogaea;* DISTRIBUTION OF MATERIALS*

	Preliminary extract	Hot water extract (gm.)	Acetone extract (gm.)	In residue (gm.)	In sample (gm.)
Total solids	....	3.8940	0.6170	....	....
Lipoids	....	0.2646	0.6170	....	0.8816
Nitrogen†	....	0.0507	....	0.6784	0.7615
Tannins, equivalent to cc. $KMnO_4$	1970.0 cc.	1245.0 cc.	0	....	....

* Weight of sample, vacuum dried, 30.1166 gm.

† Amino acid nitrogen not present in determinable amounts.

The following points with reference to table VIII are of interest. The water extract contains two substances that are believed to be of importance in the permeability of the membrane, i.e., tannins and lipoids. Tannins have been reported by REICHARD (5) to be of importance in the permeability of the barley grain to salts, and these results indicate that tannins may also take part in the permeability of the peanut seed coat to water. Remembering the effect of the acetone extractions in increasing the permeability of the membranes, it is interesting to note the low content of lipoid in this extract, only 2.04 per cent of the solid matter. A small amount seems to have great effect in reducing the rate of water movement. From the fact that lipoids were also found in the water extract we may conclude that a part of the increase after the water extraction was due to the removal of lipoids. The lack of proteins in the membrane will also be noted in the table.

Discussion

These experiments point to the important rôle of lipoids in permeability to water. The peanut seed coat, which is the most permeable of the membranes studied, is the one containing the least amount of lipoid substances, and when the lipoids are removed an increase in permeability results. Only in the case of the grapefruit did extraction with lipoid solvents fail to increase the permeability. Reasons for this behavior are suggested.

Suberized layers have usually been considered the main factors in restricting water movement through plant membranes. In these experiments there was no evidence that suberin was a dominant factor. The squash seed coat has no distinctly suberized layers, and yet it is resistant to the passage of water. These statements apply only to the membranes studied; in other membranes, more highly impermeable, suberized layers may be of first importance.

Other substances that appeared to be effective in reducing the permeability of these seed coats were tannins and pectic substances, the latter especially when deposited in thick cell walls.

Summary

1. The rôle of different substances in seed coats in regulating their permeability to water was studied.
2. Membranes were extracted with water, alcohol, acetone, ether, and calcium chloride, and their permeability measured before and after such treatments.
3. Cross-sections of the seed coats were made and tested microchemically to determine the nature of the substances present, and the effect upon them of the different methods of treatment employed in the experiment. A chemical analysis of the seed coat of the peanut and of the extracted materials was made to determine the content of tannins, lipoids, and proteins.
4. Extraction with hot water increased the permeability of the peanut and almond seed coats, the percentage increase ranging from 135 to 500 per cent. Such treatment removed from the peanut membrane the tannins and a part of the lipoid materials.

5. Extraction with hot water did not measurably increase the permeability of the grapefruit and squash seed coats; but these membranes before such treatment were so resistant to the passage of water that an increase could have resulted from the hot water extraction without being detected by the apparatus under the conditions of the experiment.

6. Extraction with hot lipoid solvents increased the permeability of all membranes studied except the seed coat of the grapefruit. The percentage of increase ranged from 15 to 871 per cent.

7. Extraction with acetone at room temperature also increased the permeability of all the seed coats except that of the grapefruit. The percentage increase ranged from 53 to 313 per cent.

8. After a membrane had its lipoid content removed, its permeability was decreased by impregnating it with the lipoid material that had been extracted; but in no case was the permeability reduced to the low point exhibited by it before the process of extraction.

9. Calcium chloride treatments increased the permeability of the membranes, but the cause of this increase could not be determined.

10. The substances found to be factors in determining the permeability of the membranes to water were lipoids, tannins, and pectic substances. Suberized layers were not found to be significant in the membranes studied, and the presence of soluble proteins could not be detected.

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